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TITLE: Vulnerary Factors to Improve Bone Healing

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Introduction

The objective for the work was to process rabbit bone specimens from the Institute of Surgical Research, follwed by sectioning and staining of the samples.

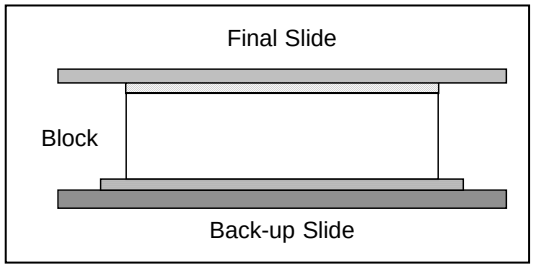
No patents application were filed.

The rabbit bone samples were received fixed in 70% alcohol. Bone was cut longitudinally (Figure 1A) and cross-sectionally (Figure 2A) on a diamond band saw and thereafter processed (dehydrated and infiltrated with xylene) according to the following schedule:

1. 70% ETOH for 4 hours
2. 80% ETOH for 2 hours
3. 95% ETOH for 6 hours
4. Fresh 95% ETOH for 4 hours
5. 100% ETOH for 6 hours
6. Fresh 100% ETOH for 4 hours
7. Xylene for 6 hours
8. Fresh Xylene for 6 hours

Xylene was then removed by incubation in Methyl Methacrylate for 3 days. Penetration of Methyl Methacrylate into the sample was achieved by incubation in infiltration media (90% Methyl Methacrylate, 10% Dibutyl Phthalate, and 70% Benzoyl peroxide 1gm/100ml) for nine days, with a change of fresh solution every three days. Samples were embedded at room temperature in a solution containing: 90% Methyl Methacrylate, 10% Dibutyl Phthalate, and 2gm/100ml 70% Benzoyl peroxide. Once bones were embedded, the blocks were trimmed down using a diamond band saw.

In order to grind and polish the slides, the blocks are adhered to a “back-up slide” (25mm x 75mm x 2mm). Blocks are then ground and polished to an even smooth surface. The smooth surface of the block is then glued to a “final” slide (25mm x 75mm) - this glue is hardened with the use of an UV light. The “final slide” is then cut away from the “back-up” slide using the diamond band saw leaving about 200-300 micrometers of the embedded sample on the “final” slide. The sample on the “final” slide is then ground down to the desired thickness and polished to a smooth even surface. The slides are then acid etched to allow the stains to penetrate the tissue.



Acid etching of slides was performed using the following procedure:

1. Incubated in 1% Formic Acid for 3 minutes.
2. Washed well in running water.
3. Sonicated in 50% ETOH for 2 minutes.
4. Rinsed in water.

The slides were then air dried before staining took place. Once dried the slides were stained with Goldner's Trichrome and Toluidine Blue stains.

The Toluidine Blue staining of the slides was performed using the following procedure:

1. Incubated in Toluidine Blue for 15 seconds.
2. Rinsed well in de-ionized water.
3. Air dried.

The Goldner's trichrome staining of the slides was performed using the following procedure:

1. Incubated in Weigert's Iron Hematoxylin for 15 minutes.
2. Rinsed in distilled water.
3. Washed gently in running tap water for 15 minutes.
4. Rinsed in distilled water.
5. Incubated in Acid Fuchsin-Ponceau for 15 minutes.
6. Rinsed in two changes of 1% acetic acid.
7. Incubated in Molybdatophosphoric acid – Orange G for 8 minutes.

8. Staining was resolved in 2 changes of 1% acetic acid.
9. Incubated in Light Green SF Yellowish for 15 minutes.
10. Staining was resolved in 2 changes of 1% acetic acid.
11. Rinsed in water.
12. Air dried.

**Figure 1**

**1A**

Longitudinal section- fresh bone sample

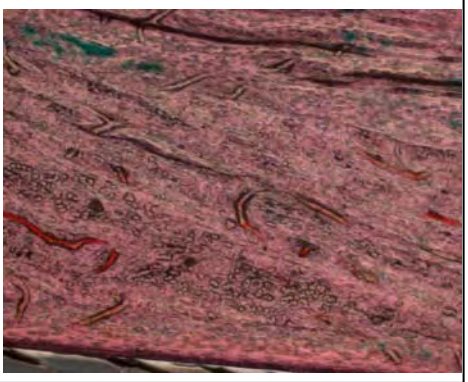
**1B**

Trichrome- longitudinal section- 5X

**1C**

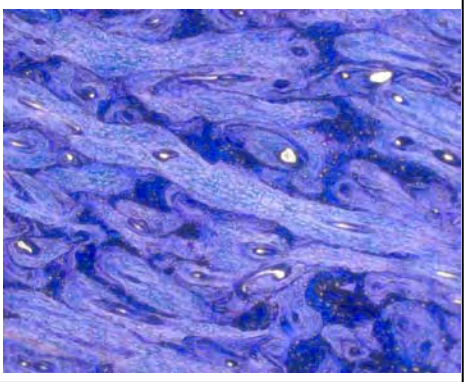
Toluidine Blue- longitudinal section- 5X

**1D**



Trichrome- longitudinal section- 10X

**1E**



Toluidine Blue-longitudinal section-10X

**Figure 2**

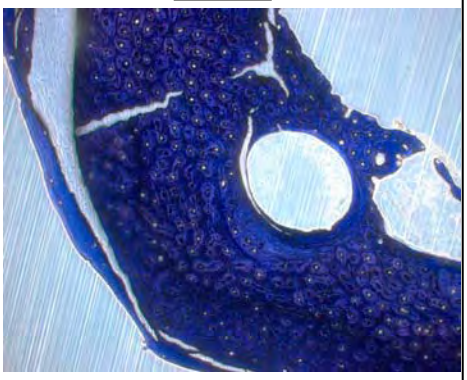
**2A**

Cross-section- fresh bone sample

**2B**

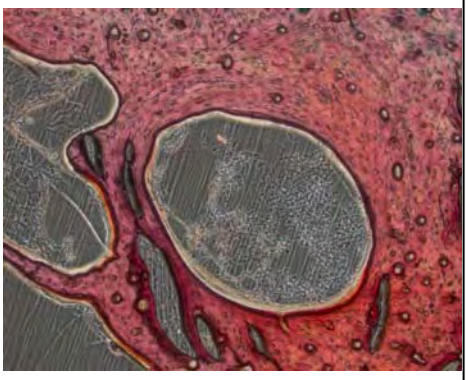
Trichrome- cross-section- 5X

**2C**



Toluidine Blue- cross-section- 5X

**2D**



Trichrome- cross-section- 10X

**2E**

Toluidine Blue- cross-section-10X